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THE EFFECT OF REDUCING THE QUANTITY OF EGG YOLK IN BOVINE SEMEN DILUENTS

BY

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MINISTRY OF AGRICULTURE AND FISHERIES, READING

The extensive use of artificial insemination in cattle breeding has been due to the evolution of satisfactory semen diluents, which enabled comparatively large numbers of cows to be served from each ejaculate with little alteration in the fertilizing capacity of the semen. Probably the greatest single advance in semen diluents to date was the introduction of egg yolk by Phillips and Lardy (1940), who recommended the use of a diluent containing equal parts of a phosphate buffer and egg yolk. Salisbury *et al.* (1941) reported that a more easily prepared sodium citrate buffer was equally effective when mixed with equal parts of egg yolk by volume. The value of egg yolk in semen diluents is not at all well understood, although it would appear to have a protective action and several workers have demonstrated that its addition protects spermatozoa from the effects of cooling and temperature shock.

Swanson (1949) investigated the effect of varying proportions of egg yolk and sodium citrate buffer in bull semen diluents upon sperm motility. These experiments showed that motility was maintained equally well on storage when a diluent containing 20 per cent. egg-yolk was compared with the normal diluent containing 50 per cent. egg-yolk. It was also shown that reducing the egg-yolk to 10 per cent. had no apparent effect on its protective action during cooling or against cold shock. No information was given on the effects of varying the proportion of egg-yolk in the diluent on fertility except that at the University of Tenessee a diluent containing a reduced proportion of egg-yolk was used successfully in routine inseminations over a period of 12 months ; however, no data was given on this work.

During a series of investigations on semen diluents at the Reading Cattle Breeding Centre observations were included on the reduction of the proportion of egg-yolk in the diluent and this will be described as it may be of practical interest.

MATERIALS AND METHODS

The buffer used in this investigation consisted of 3 per cent. sodium citrate ($Na_3C_6H_5O_7 . 2H_2O$) in sterile glass-distilled water, instead of the usual 3·6 per cent. solution. According to the investigation of Swanson (1949) and Salisbury *et al.* (1948) the 3 per cent. solution was nearer to the point of isotonicity of bovine semen. The control diluent therefore contained equal parts of egg-yolk and 3 per cent. citrate buffer, and the trial diluent one part of egg-yolk and three parts of the same citrate buffer.

The usual laboratory storage tests were set up ; equal volumes from each of five semen samples were diluted 1 in 20, and 1 in 50 with the control and trial diluents. These samples were stored in the laboratory refrigerator at 4° to 6° C. and examined for motility every three days.

In the fertility trial equal volumes of diluted semen were prepared from each ejaculate using the control and trial diluents. The extent of dilution was 1 in 50, or 1 in 20, according to the breeding history of the bull and the quality of the semen on microscopic examination at the time of collection ; about 83 per cent. of the semen used was diluted 1 in 50. To ensure that approximately equal numbers of unselected cows were inseminated by the trial and control diluents, these diluents were used by each inseminator on alternate days. This ensured that they were both used by all inseminators and that they were distributed evenly throughout the area served by the centre. The conception rate was estimated as the percentage of first inseminations not reported to have returned to service within a period of 16 weeks from the date of insemination. Semen was collected three times weekly and used for two days except at week-ends, when three-day-old semen was used on Sundays.

RESULTS

After 13 days' storage in the laboratory refrigerator there was no significant difference between the control and the trial diluents. These results confirmed the findings of Swanson (1949) and so it was considered that further storage tests were unnecessary. The fertility trials were then proceeded with and the results are summarised in Table I.

TABLE I

	CONTROL DILUENT		TRIAL DILUENT	
Day of Use	No. of First Inseminations	Conception Rate	No. of First Inseminations	Conception Rate
1st	889	61·3%	805	63·2%
2nd	828	65·0%	822	60·7%
3rd	168	54·8%	167	53·3%
Total	1,885	62·3%	1,794	61·1%

There was no significant difference in the fertilizing capacity of semen diluted with the trial or control diluents on any day of use or in the total results when the above figures were subjected to the chi-square test. There was, however, a significant drop in conception rate on the third day of use, which was common to both groups.

The fertility level obtained during the investigation may be considered lower than that generally obtained in artificial insemination. This reduction was attributable to one bull, which had been used extensively. The animal concerned had a long history of high fertility and without any observed change in the semen quality its fertility became low during this period. If the inseminations done with this semen are excluded the general level of fertility is satisfactory as shown in Table II.

TABLE II

Day of Use	Control Diluent: No. of First Inseminations	Control Diluent: Conception Rate	Trial Diluent: No. of First Inseminations	Trial Diluent: Conception Rate
1st	770	66·8%	701	67·8%
2nd	731	68·9%	697	65·3%
3rd	141	59·6%	132	60·8%
Total	1,642	67·1%	1,530	66·0%

In practice it was found that the individual spermatozoa were more clearly visible on microscopic examination when the reduced egg-yolk diluent was used. Also, it was noticed that efficient inseminating syringes were necessary as the diluted semen was less viscous and leakage from the insemination pipettes was more liable to occur.

DISCUSSION AND SUMMARY

This investigation confirms the *in vitro*-work done by Swanson and demonstrates that under ordinary field conditions a reduction by 50 per cent. of the egg-yolk in the yolk-citrate diluent does not interfere with the fertilizing capacity of diluted bovine semen. The change in the consistency of the semen by reducing the proportion of egg-yolk had no apparent effect on the keeping quality of the semen nor had the removal of some of the protective material provided by the egg-yolk.

The introduction of the trial diluent containing 25 per cent. of egg-yolk into the routine work of the Reading Centre would mean a saving of about 260 dozen eggs or £50 per annum. Over a large number of artificial insemination centres this saving would become significant.

The authors consider it would be advisable for other artificial insemination centres to repeat this work, as the result of this investigation may be of practical value in the economic operation of a centre.

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PREGNANCY DIAGNOSIS TESTS IN FARM ANIMALS (USING THE COMMON INDIAN TOAD, *BUFO MELANOSTICTUS* SCHNEID)

BY

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A reference to Cowie's recent publication[3] will show clearly that no successful pregnancy tests of any diagnostic value have been found in any farm animal excepting the mare, relative to the hormonal investigations into such body fluids as urine, blood (serum), saliva and milk. Since the publication of this book and within the past three years an entirely new test—the male Salientian (frogs and toads) test—has been instituted and has stimulated a brisk investigation into this field in both human and veterinary medicine, more especially because of its very encouraging attributes in regard to speed of reaction, accuracy, sensitiveness and economy. Following success by the use of male Salientian tests in human medicine, a few attempts have been made on veterinary subjects, but negative results have so far been reported from the urine and serum tests[1-4], although Neto[5] claimed to have obtained some apparent success in mares. The present writer, in collaboration with his student, Mr. Bardhan[1], has been able to show recently that the pregnancy diagnosis test is possible in cows from processed faecal solution, utilizing the common Indian male toad, *Bufo melanostictus*, as the test animal. It was reported in their preliminary note that diagnosis could be made in cows from the 55th day of pregnancy, but their subsequent studies on a further series of tests run at this laboratory detected pregnancy as early as the 22nd day of gestation, and even in one instance at only 15 days gestation (unpublished data). To the best of my knowledge no actual demonstration of this gametokinetic reaction of faecal solution in the male Salientia has been reported before. Encouraged by this finding, experiments on similar lines were designed by me in collaboration with my students, Messrs. S. L. Bose and H. Kundu, for the investigation of pregnancy diagnosis in buffaloes and goats. It may be noted that in these two species no successful pregnancy test has been described, although Csiki claimed to have obtained positive results from the Cuboni test with the urine of buffaloes after the 100th day of pregnancy (*vide* Cowie[3]).

BUFFALO: Success with the cows warranted an expectation of a successful diagnostic pregnancy test in buffaloes. But this expectation was not fulfilled. The exact procedure as adopted in the cow test was found unsuitable, since it always gave a negative result. After a protracted trial, however, the following modification in the procedure was adopted and found suitable for the present.

The urine-free faeces of buffaloes were collected from local private cattle pens where buffaloes are kept and bred. The period of pregnancy was calculated from the dates of their services as reported by their owner. Twenty-five grammes of faeces were mixed with 100 c.c. of distilled water and kept overnight in the frigidaire. On the following day the solution was centrifuged and filtered in a manner suitable for injection into the toad without any apparent detrimental effect on it during experimentation. Three to four successive doses of 5 c.c. each of this processed solution were administered subcutaneously into the lymph sac of the male toad at intervals of half an hour. Before each

injection was given, the samplings of toad's urine were made available for examination under the microscope for the presence of sperms, the process being continued for two hours after the last injection was given. Generally at the end of the fourth injection a positive reaction was evoked. The end result was very clear, *viz.*, the presence of sperms in the voided urine. Other details of procedure were more or less the same as in the case of the cow described previously[1]. It was possible in this way to diagnose pregnancy in buffaloes from as early as the 35th day.

Broadly speaking, the main difference between the cow and buffalo pregnancy tests lies in the quantity of faecal matter used and the time taken for processing the solution. It took a couple of days to run the test successfully in the case of the buffalo. Such a discrepancy in the matter of processing the solution cannot be explained at present. Nevertheless, the gametokinetic hormonal reaction of the solution in the toad is the same as that in the cow. In the case of the buffalo, however, the hormone titre seems to be at a slightly lower level than that of the cow. From our preliminary investigation it would appear that the diagnostic pregnancy test in the buffalo, relative to the uses of faeces and the male toad, as the suitable material and animal respectively, might prove of value. The results obtained from ten different buffalo pregnancy cases seem so far to be reassuring in respect of accuracy and economy. A full report of the work is in the course of preparation by the signatory and his student, Mr. S. L. Bose, and soon will be published elsewhere.

GOAT: While a 100 per cent. success was obtained in buffaloes, only a few sporadic positive results were achieved in a series of more than 50 tests in goats. Here again goat faeces were used for processing the solution in much the same way as in cows and buffaloes. Three, 5 and 10 grammes of faeces were tried. Each quantity yielded both positive and negative results on different days, the latter being more in evidence. It was pointed out in a previous note[1], on the basis of Turner's statement, that there is slightly more gonadotrophic hormone activity in goat's faeces than in the cow's. The present studies, however, tend to indicate not only a low hormone titre but the capricious nature of hormone excretion in the goat's faeces. Nevertheless, the mere fact that positive reactions could be evoked from processed faecal solution in a few cases of both early and late pregnancy is encouraging enough to justify a continuation of this work. It is hoped that the faecal solution, as such, with different proportions of faecal material or its extraction, ultimately may prove this test to be of diagnostic value in this animal.

Although I have not dealt with other farm animals for which no adequate pregnancy diagnosis tests are available, my preliminary studies on faecal matter as a suitable test material seem to promise well for future researches in this line. Unfortunately, I could make no attempt to pursue them owing to the fact that in Calcutta such breeding farm animals as the horse, sheep, etc., are not available for the purpose. The present author thinks that gonadotrophic hormonal tests based on faecal material should be given a fair trial for pregnancy diagnosis in all those farm animals which have so far yielded either negative or unconfirmed positive results from experiments with such body fluid test material as serum, urine, milk, sweat, etc. The simplicity of the technique, as much as its economy, renders the tests of real value.

Acknowledgment.—The collaboration of my students, Messrs. S. L. Bose, H. Kundu and N. R. Bardhan, in this study is gratefully acknowledged.

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DISEASES OF ANIMALS ACTS, 1894 TO 1937, AND AGRICULTURE ACT, 1937 (PART IV).

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Period	Anthrax	Foot-and-mouth	Fowl Pest	Parasitic Mange*	Sheep Scab	Swine Fever
Sep. 16th to 30th, 1950	12	—	—	—	1	48
Corresponding period in—						
1949	16	—	42	—	—	—
1948	9	1	2	—	9	—
1947	11	—	101	—	7	3
Jan. 1st to Sep. 30th, 1950	277	20	102	—	21	176
Corresponding period in—						
1949	166	13	408	—	33	5
1948	79	2	249	1	48	23
1947	87	103	861	1	64	29

NOTE.—The figures for the current year are approximate only.

* Excluding outbreaks in Army Horses.

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